Drug-induced autoimmunity

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Introduction

The phenomenon of drug-induced autoimmune diseases has frequently been reviewed since the first sulfadiazine-induced case of systemic lupus erythematosus (SLE) was recognised by Hoffman in 1945.[1–3] At first, the reports were sporadic, but gradually specific groups of drugs with the potential to induce SLE emerged. The toxicological significance of this form of immune disregulation, and the need for methods allowing hazard identification has been further emphasised by epidemics such as Spanish Toxic Oil Syndrome and Eosinophilia-Myalgia Syndrome, which both share features of systemic autoimmune disorders.[4]

In trying to analyse drug-induced autoimmune reactions from a toxicological point of view the following questions need to be addressed:

(i) are there particular chemical or pharmacological properties which endow some low molecular weight (LMW) compounds with the potential to provoke autoimmune reactions?
(ii) can drug-induced autoimmune disorders be reproduced in animals?
(iii) which methods or parameters allow hazard identification?
(iv) do drugs actually induce autoimmune disorders *de novo* or by activation of a predisposition, or do they act by a combination of both mechanisms, and if so, what makes some individuals more susceptible to the development of these disorders than others?

Chemical and pharmacological aspects

A large variety of drugs, with a molecular weight of less than 1000 dalton, have been associated with induction of autoimmune disorders in susceptible individuals.[1–3] These drugs belong to different chemical classes and include among others derivatives of aromatic amines, hydrazines, hydantoins, thioureylenes, oxazolidinediones, succinimides, dibenzazepines, phenothiazines, sulfonamides, pyrazolones, amino acids, allyl amines, furthermore halothane, mercuric chloride, and gold preparations. With few exceptions, these compounds are heterocyclic and many contain at least one aromatic group, suggesting that particular chemical entities may favour induction of immune disregulation.

From a pharmacological point of view, the majority of autoimmune disease-inducing drugs can be ranked among β-adrenergic-receptor-blocking compounds, drugs acting at the central nervous system (CNS), anti-thyroidal agents, and anti-infectious agents. In view of the tight functional connectivity between immune, nervous and endocrine systems, which is, at least partially, effected by shared receptors and mediators among the systems, it is a possibility that CNS drugs modulate immune responses by acting at these receptors or inducing common mediators.

Animal models of drug-induced autoimmune reactions

Most attempts to induce autoimmune disorders with drugs in experimental animals have been unsuccessful,[2] and presently only few reproducible models of drug-induced autoimmune disease are available. Among these, glomerulonephritis induced by mercuric chloride-, gold-containing compounds and D-penicillamine in inbred Brown Norway rats and B10.S mice have been best studied.[5] Mechanistically, it has been proposed that drugs may elicit immune responses analogous to graft-versus-host (GVH) reactions[5] and that formation of reactive metabolites in neutrophils and monocytes could be linked to the induction of immune disregulation.[3]

Diphenylhydantoin (DPH), a drug which has been studied in great detail in this regard both in the laboratory and in the clinic, is able to induce immunodeficiency, lymphoproliferation and autoimmune reactions in man.[6] Notably IgA deficiency and impairment of delayed-type hypersensitivity, T-cell mitogen responsiveness and T-helper cell function, point to the immunosuppressive action of DPH. Other side-effects reveal the capacity of DPH to elicit a spectrum of lymphoproliferative disorders at low incidence, including lymphoma, lymphadenopathy, and autoimmune diseases such as SLE, scleroderma and vasculitis.[6] Animal experiments have confirmed some of the immunosuppressive actions of DPH in man. In mice, in particular, suppression of antibody formation, natural killer cell activity, and cytotoxic T-lymphocyte function have been found. It has been shown that DPH can cause atrophy of lymphoid organs as well as lymphoproliferation in mice.[6] Three strains of female

mice differing in spontaneous lymphoma incidence, i.e. C3Hf mice (H-2^k; resistant), C57BL mice (H-2^b; low spontaneous incidence), and SJL mice (H-2^s; high spontaneous incidence) exposed to DPH (approximately 40 mg DPH/kg/day) via liquid diet starting at week 8 to 12 of age, developed different patterns of lymphoproliferative lesions.[6] In C57BL/6-*lpr/lpr* mice that spontaneously develop lymphoproliferative disease and SLE-like symptoms, DPH was shown to have beneficial effects by reducing lymphadenopathy and autoantibody levels.[7] Thus, animal studies enable recognition of the immunosuppressive and lymphoproliferative activities of DPH as observed in man, but fail to demonstrate the autoimmunising potential of DPH. In an attempt to find an animal model for toxic oil syndrome, fatty acid anilides administered to B10.S mice induced significant increases of serum IgE, IgM and IgG1 levels, autoantibodies, as well as mRNA encoding IL-1β and IL-6 in splenocytes.[8]

Hazard identification in toxicological testing: detection of immune disregulation

Currently, there are no predictive assays developed and validated to identify the potential of drugs to induce systemic hypersensitivity or autoimmune responses in the early phases of drug development. These side effects usually become manifest only during advanced clinical development. The conditions used in routine preclinical toxicological screening are obviously not optimal for the detection of the immune-disregulating potential of drugs and chemicals (e.g. small animal number, use of outbred animal strains, dynamics of disease development versus snapshot determinations, lack of predictive parameters). An economically and practically relevant question concerning screening studies is whether actual evidence of an agent's ability to induce manifest hypersensitivity or autoimmune disease should be and can be obtained, or whether (preferably short-term) assays not measuring the actual clinical endpoints can be sufficiently predictive in this respect. As it has become clear that T-cells are primary players in the initiation and perpetuation of spontaneous as well as induced systemic autoimmune disorders[5] an important aspect to study in toxicology is whether a drug's ability to cause T-cell activation is predictive of its ability to cause allergic and/or autoimmune side effects.

In addition to morphological examinations in routine toxicological studies, measurements of some immunologically relevant serum parameters can provide important information about antibody-mediated responses. Parameters may comprise levels of total immunoglobulins and of various immunoglobulin (sub)classes, immune complexes and some commonly observed autoantibodies, e.g. antinuclear (ANA), anti-histone and anti-single-stranded (denatured; ssDNA) autoantibodies. Some of these autoantibodies proved to be useful in the diagnosis of procainamide-induced SLE in man.[4] Antinuclear antibodies in patients with procainamide-induced SLE appeared to be of the IgG class (in particular IgG1 and IgG3), whereas IgM antinuclear antibodies are predominant in asymptomatic users of the drug.[9]

Routine methods for monitoring and predicting cell-mediated autoimmune reactions in toxicity studies are virtually lacking. A parameter to monitor human T-cell activation nonspecifically could be the soluble interleukin-2 receptor (sIL-2R; p55 chain).[10] Although both T-cells and macrophages shed sIL-2R upon activation, its levels in serum and to a lesser extent in urine paralleled various kinds of T-cell-mediated immune processes in man. Since sIL-2R can be measured by ELISA, it could fit very well into routine toxicological procedures, but its relevance in animal models has still to be assessed.

It remains to be established whether cytokine profile measurements will become useful tools in drug safety studies. Various cytokines are involved in the induction and development of autoimmune diseases.[5,10] This indicates that Th_1 or Th_2 T-helper cells may be the dominant mediators in a particular disease.[5,10] While such studies provide valuable insight in the etiology of the different diseases, it is to be questioned, whether such detailed data need to be obtained in routine toxicity studies. An additional problem is that levels of many cytokines in the circulation as well as lymphoid organs are low or undetectable, even when symptoms are clearly manifest such as in GVH disease.[11] A clear exception is IL-6 that is profoundly elevated in serum and/or tissue in many inflammatory conditions.[11]

Based on the hypothesis that chemicals may elicit autoimmune disorders by a mechanism resembling GVH reactions, an existing GVH assay, the popliteal lymph node assay (PLNA), has been adopted to study chemical-induced immune reactions.[12] The PLNA seems to be a versatile tool to recognise T-cell-activating drugs and chemicals, including autoimmunogenic chemicals. However, further mechanistic studies and inter-laboratory validation is required, before the assay can be recommended for routine use in preclinical toxicity screening.

Multifactorial etiology: difficulties for risk assessment

The spectrum of factors associated with autoimmune diseases has been extensively reviewed and appropriately termed *The Mosaic of Autoimmunity*'.[13] The concerted action of individual factors and the chemical appear to somehow determine whether or not autoimmune disease will develop. Risk assessment ideally requires knowledge of all endogenous as well as exogenous factors that influence susceptibility to drug-induced unto-

ward immune reactions. Factors having major influence on immune reactions are becoming more defined, and include polymorphisms of MHC and metabolism genes, and, moreover, genetic variation in the magnitude of glucocorticoid responses.[14] Although a drug's potential to cause autoimmune disease may reflect its intrinsic chemical or pharmacological characteristics, the underlying disease necessitating treatment may be at play as well. For instance, drugs used to treat bacterial infections, like penicillins, sulfonamides and nitrofurantoin are relatively frequent inducers of immunological side effects. Since infectious agents themselves have been implicated in the etiology of autoimmune diseases, this may lead to a biased implication of these drugs in the diseases. On the other hand it is not unlikely that sensitisation of T-cells to the drugs is stimulated by infection, for instance, as a consequence of the adjuvant action of cytokines generated during infection. Another possibility could be, that T-cells, which contribute to the normal or drug-induced autoreactive T-cell pool, can be activated by microbial superantigens produced as a result of microbial growth after drug-induced immunosuppression, resulting in the induction or exacerbation of autoimmune reactions.

Conclusions

Hazard identification of the autoimmune disease-inducing potential of a given compound in routine toxicology, as currently practiced, will remain difficult in the near future. Because drugs may interact with the immune system at different levels, it is unlikely that one assay or methodology will be able to predict the autoimmunising potential of the various classes of drugs. To establish adequate parameters which allow hazard identification, further research into the mechanisms of drug-induced autoimmune reactions is required. A major challenge for the development of predictive toxicity testing methods and risk assessment represents the analysis of the contribution of individual factors to the development of disease.

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